AD NUMBER

AD808017

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; JAN 1967. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Releases Branch, Frederick, MD 21701.

AUTHORITY

Army Biological Defense Research Lab ltr dtd 28 Sep 1971

AD 808017

TECHNICAL MANUSCRIPT 343

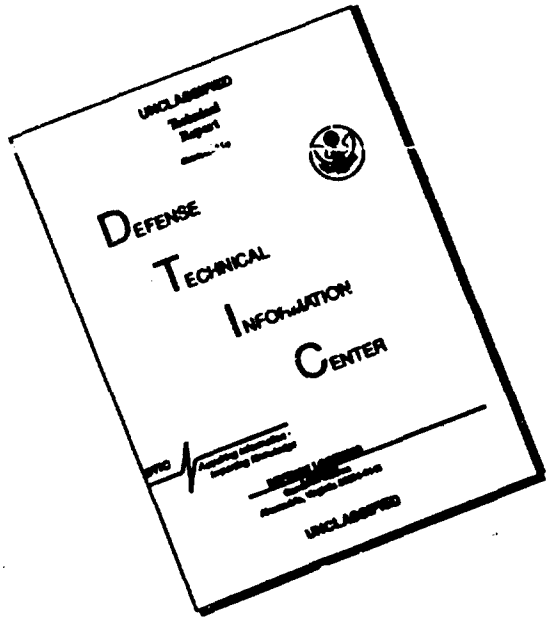
SUPPRESSION OF VIRULENCE IN PASTEURELLA PESTIS BY THE EPISOME F-LAC

William D. Lawton
Robert M. Zsigray
Michael J. Surgalla

JANUARY 1967

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 343

SUPPRESSION OF VIRULENCE IN *PASTEURELLA PESTIS* BY THE EPISOME F-*LAC*

William D. Lawton

Robert M. Zsigray

Michael J. Surgalla

Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORY

Project 1C014501B71A

January 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

The episome F-*lac* was transferred from *Escherichia coli* F-*lac* into virulent strains of *Pasteurella pestis*. The resulting strains of *P. pestis* F-*lac* were avirulent in mice but segregated (F^-)lac^- clones that were fully virulent. This suppression of virulence by F-*lac* was correlated with a concomitant suppression of the calcium requirement, which is known to be associated with virulence in *P. pestis*.

I. INTRODUCTION

The episome F-lac has been transferred between Escherichia coli and species of Salmonella, Shigella, Proteus, Serratia, and avirulent strains of Pasteurella.[1] Evidence is presented here that this episome suppresses the expression of the calcium requirement known to be associated with virulence in P. pestis.

The abbreviations used in this manuscript are: lac, lactose; phe, phenylalanine; cys, cysteine; met, methionine; val, valine; ile, isoleucine; gly, glycine; try, tryptophan; pro, proline; his, histidine.

II. MATERIALS AND METHODS

A. STRAINS

Salmonella typhosa try$^-$ F-lac, S. typhosa try$^-$ F^{s}lac, and S. typhosa try$^-$ R$^+$ were obtained from Dr. S. Falkow. Escherichia coli K-12 F-lac strain 23.10.S was obtained from Dr. R. C. Clowes. Other strains used, obtained from our stock collection, were E. coli K-12 pro$^-$, Pasteurella pseudotuberculosis type I try$^-$, Pasteurella pestis strains Saka, Alexander, and 360, and a methionine-independent histidine-requiring auxotroph from strain Alexander.

B. MEDIA

Minimal lactose agar medium (designated 1 L) was prepared as follows [final concentration (w/v) in parentheses]. Bacto agar (1.5%) plus 10 ml of a 100 X stock mixture of $MgSO_4 \cdot 7H_2O$ (0.01%), $ZnSO_4 \cdot 7H_2O$ (0.00001%), $MnSO_4 \cdot H_2O$ (0.00001%), NH_4Cl (0.053%), and $(NH_4)_2SO_4$(0.053%) plus 905 ml of distilled water were autoclaved and the following additions made aseptically: 10 ml of 1.0 M potassium phosphate buffer, pH 7.2; 10 ml of a filtered 8 mg/ml stock solution of $FeCl_3$ (0.008%); 50 ml of 20% lactose (1.0%). This provided 1 liter of medium. Amino acids (usually 0.005%) were added as required. A mixture of six amino acids was necessary for the growth of typical P. pestis: phe (0.01%), cys (0.005%), met (0.005%), val (0.005%), ile (0.005%), and gly (0.005%). This medium is similar to the hemin medium used by Jackson and Burrows.[2]

A complete lactose-indicator medium (BAB-*lac*) was obtained by supplementing Difco blood agar base with lactose (1%), brom thymol blue (0.0025%), and triphenyl tetrazolium chloride (0.005%). *Lac*$^+$ colonies were yellow and *lac*$^-$ colonies were red on this medium.

C. F-*LAC* TRANSFER

Overnight shake cultures in Difco heart infusion broth (HIB) of donor and recipient strains were usually mixed together for 2 to 4 hours and then plated at appropriate dilutions on a minimal lactose medium formulated to permit only *lac*$^+$ recipient cells to form colonies (medium 1 L plus the amino acids required for growth of the recipient). This method was used to pass F-*lac* from *S. typhosa try*$^-$ F-*lac* into *E. coli* K-12 *pro*$^-$ and subsequently from *E. coli* K-12 *pro*$^-$ F-*lac* into *P. pestis* Saka and Alexander. In order to obtain an estimate of F-*lac* transfer frequency, the method was altered as follows. Approximately 10^8 cells of the recipient *lac*$^-$ strain were spread on a minimal lactose agar medium formulated to permit only *lac* recipient cells to form colonies. The donor F-*lac* strain was diluted in 0.03 M potassium phosphate buffer, pH 7.2, and a single drop (0.05 ml) of each dilution was spotted on top of the recipient lawn and, as a control, on a sterile plate of the same medium. In this manner, F-*lac* was transferred from *E. coli* K-12 F-*lac* 23.10.S to *P. pseudotuberculosis* type I *try*$^-$ and subsequently from *P. pseudotuberculosis* type I *try*$^-$ F-*lac* into *P. pestis* strain 360 (*cys*$^-$*phe*$^-$*his*$^-$).

D. ASSAY PROCEDURES

Carbohydrate fermentation and urease production were measured by inoculating a suspension of the test organisms into Difco purple broth supplemented with 1% of the desired carbohydrate and into Difco urea broth. Standard methods were used for the detection of virulence,[3] specific antigens,[4] pigmentation,[3] calcium requirement[3] and the production of pesticin, fibrinolytic factor, and coagulase.[5]

III. RESULTS

The episome F-*lac* transferred from *E. coli* K-12 *pro*$^-$ F-*lac* to two virulent strains of *P. pestis*, Alexander and Saka, at an estimated frequency between 10^{-3} and 10^{-4} per donor cell. *P. pestis* F-*lac* clones were purified and several of the purified clones and their (F$^-$)*lac*$^-$ spontaneous segregants were tested for virulence in mice (Table 1). Based on ten single *lac*$^+$ isolates and nine single *lac*$^-$ isolates derived from them, we observed a complete correlation between the presence of F-*lac* and the phenotypic suppression of virulence. All of the nine *lac*$^-$ segregants derived from these phenotypically avirulent F-*lac* isolates were fully virulent.

These data, plus supporting data obtained with two different virulent strains of P. pestis, MP6 and an arginine-requiring auxotroph of Alexander, prompted us to study the correlation in more detail. P. pestis strain 360 was infected with F-lac from P. pseudotuberculosis type I try$^-$ F-lac at a frequency of 10^{-3} per donor cell. Fourteen separate lac$^+$ colonies were purified, tested, and found to be negative for virulence, urease production, and fermentation of rhamnose and melibiose. The latter three properties are characteristically negative in P. pestis and positive in P. pseudotuberculosis. Satisfied that we could pass F-lac into strain 360 and suppress virulence, we streaked a single colony of strain 360 F-lac five times successively on the selective agar medium and twice on BAB-lac to insure adequate purification. A single lac$^+$ colony from the final streaking was then streaked on BAB-lac medium and a spontaneous lac$^-$ segregant clone was picked. A comparison of 360 F-lac, 360 (F$^-$)lac$^-$, and the original parent strain 360 is presented in Table 2 (virulence) and Table 3 (other characteristics). The suppression of virulence by F-lac was demonstrated as well as the restoration of virulence upon loss of F-lac. Of the 24 properties tested, only the calcium requirement was affected by the presence of F-lac.

TABLE 1. SUPPRESSION OF THE VIRULENCE OF P. PESTIS IN MICE BY THE EPISOME F-LAC

P. pestis Strain	No. of Individual Colonies Tested	No. Deaths/Total at Dose			
		2×10^7	2×10^5	2×10^3	2×10^1
Alexander F-lac	6	2/25	0/30	0/30	0/29
Alexander (F$^-$)lac$^-$	6	30/30	30/30	30/30	25/30
Saka F-lac	4	0/20	0/20	0/20	0/20
Saka(F$^-$)lac$^-$	3	15/15	15/15	15/15	14/15

TABLE 2. SUPPRESSION OF THE VIRULENCE OF P. PESTIS STRAIN 360 IN MICE BY THE EPISOME F-LAC

Strain 360 (parent)		Strain 360 F-lac		Strain 360 (F^-)lac^-	
Dose	No. Dead/Total	Dose	No. Dead/Total	Dose	No. Dead/Total
358	10/10	4×10^8	2/5	27,500	5/5
107	7/10	4×10^7	0/5	2,750	4/5
21	5/10	4×10^6	0/5	275	4/5
5	1/10	4×10^5	0/5	255	4/5
1	0/10	4×10^4	0/5	66	4/5
		4×10^3	0/5	27	3/5
		4×10^2	0/5	14	1/5
				7	0/5

TABLE 3. EFFECT OF THE F-*LAC* EPISOME ON THE CHARACTERISTICS OF *P. PESTIS*

Property	*P. pestis* Strains		
	360 (parent)	360 F-*lac*	360 (F^-)*lac*$^-$
Production of:			
fraction I antigen	+	+	+
toxin antigen	+	+	+
V antigen	+	+	+
W antigen	+	+	+
pesticin I	+	+	+
fibrinolytic factor	+	+	+
coagulase	+	+	+
pigmentation	+	+	+
urease	-	-	-
Sensitivity to:			
phages T7 and C16	+	+	+
phages T1, T2, T3, T4, T5, T6	-	-	-
Utilization of:			
lactose	-	+	-
melibiose	-	-	-
glycerol	-	-	-
rhamnose	-	-	-
xylose	+	+	+
Growth requirements:			
amino acids	*cys,phe,his*	*cys,phe,his*	*cys,phe,his*
Ca^{++} at 37 C	+	-	+

Transfer of F'*lac* or R factor into *P. pestis* strain 360 was accomplished, but the resulting episome-containing isolates of *P. pestis* showed no suppression of virulence or loss of the calcium requirement.

IV. DISCUSSION

The suppression of virulence by F-lac should be of interest to plague investigators because it may provide a useful technique to understand virulence better in this pathogen. Our observation supports the opinion of Brubaker and Surgalla[6] concerning the importance of the calcium requirement as a virulence factor. Since the metabolic basis for the calcium requirement is unknown, speculation on possible mechanisms for the suppression of virulence is difficult.

A puzzling aspect of these data is the fact that large numbers of P. pestis F-lac organisms usually did not kill any mice, even though most of the F-lac isolates spontaneously segregated 0.1% to 1.0% lac$^-$ clones, which are virulent and would be expected to kill the mice in the absence of F-lac cells. Several explanations are possible: (i) the F-lac strains did not segregate in vivo; (ii) the suppression of virulence may be due to the production of a soluble substance that can diffuse into (F$^-$)lac$^-$ cells and suppress virulence; or (iii) the presence of a large excess of F-lac phenotypically avirulent cells may create an immune response in the animals soon enough to prevent the expression of virulence by the (F$^-$)lac$^-$ cells.

From a genetic viewpoint, our observation may lead to further knowledge concerning functions of the F factor. Both F^{2}lac[7] and the R factor used in this study are related to the F factor but deficient in their ability to promote chromosomal transfer. Since these two factors did not suppress virulence, it is possible that the suppression of virulence depends on one of the functions of the F factor required for chromosome transfer, such as the ability to attach to the chromosome.

LITERATURE CITED

1. Marmur, J.; Falkow, S.; Mandel, M. 1963. New approaches to bacterial taxonomy. Annu. Rev. Microbiol. 17:329-372.

2. Jackson, S.; Burrows, T.W. 1956. The pigmentation of Pasteurella pestis on a defined medium containing haemin. Brit. J. Exp. Pathol. 37:570-576.

3. Higuchi, K.; Smith, J.L. 1961. Studies on the nutrition and physiology of Pasteurella pestis: VI. A differential plating medium for the estimation of the mutation rate to avirulence. J. Bacteriol. 81:605-608.

4. Lawton, W.D.; Fukui, G.M.; Surgalla, M.J. 1960. Studies on the antigens of Pasteurella pestis and Pasteurella pseudotuberculosis. J. Immunol. 84:475-479.

5. Brubaker, R.R.; Surgalla, M.J.; Beesley, E.D. 1965. Pesticinogeny and bacterial virulence. Zentralbl. Bakteriol. Parasitenk. Infekt. Hyg. 196:302-315.

6. Brubaker, R.R.; Surgalla, M.J. 1962. Genotypic alterations associated with virulence in streptomycin-resistant Pasteurella pestis. J. Bacteriol. 84:615-624.

7. Falkow, S.; Baron, L.S. 1962. Episomic element in a strain of Salmonella typhosa. J. Bacteriol. 84:581-589.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY *(Corporate author)*
Department of the Army
Fort Detrick, Frederick, Maryland, 21701

2a REPORT SECURITY CLASSIFICATION
Unclassified

2b GROUP

3 REPORT TITLE
SUPPRESSION OF VIRULENCE IN PASTEURELLA PESTIS BY THE EPISOME F-LAC

4 DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5 AUTHOR(S) *(Last name, first name, initial)*
Lawton, William D.
Zsigray, Robert M.
Surgalla, Michael J.

6 REPORT DATE	7a TOTAL NO. OF PAGES	7b NO. OF REFS
January 1967	12	7

8a CONTRACT OR GRANT NO.	9a ORIGINATOR'S REPORT NUMBER(S)
b PROJECT NO. 1C014501B71A	Technical Manuscript 343
c.	9b OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10 AVAILABILITY/LIMITATION NOTICES
Qualified requesters may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	Department of the Army Fort Detrick, Frederick, Maryland, 21701

13 ABSTRACT

The episome F-lac was transferred from Escherichia coli F-lac into virulent strains of Pasteurella pestis. The resulting strains of P. pestis F-lac were avirulent in mice but segregated (F^-)lac^- clones that were fully virulent. This suppression of virulence by F-lac was correlated with a concomitant suppression of the calcium requirement, which is known to be associated with virulence in P. pestis.

14. Key Words

*Pasteurella pestis
*Virulence
*Episomes
Calcium
Escherichia coli
Culture media
Conjugation

DD FORM 1 JAN 64 1473

Unclassified
Security Classification